Effective Shortening in Length of Glycolipid Nanotubes with High Axial Ratios

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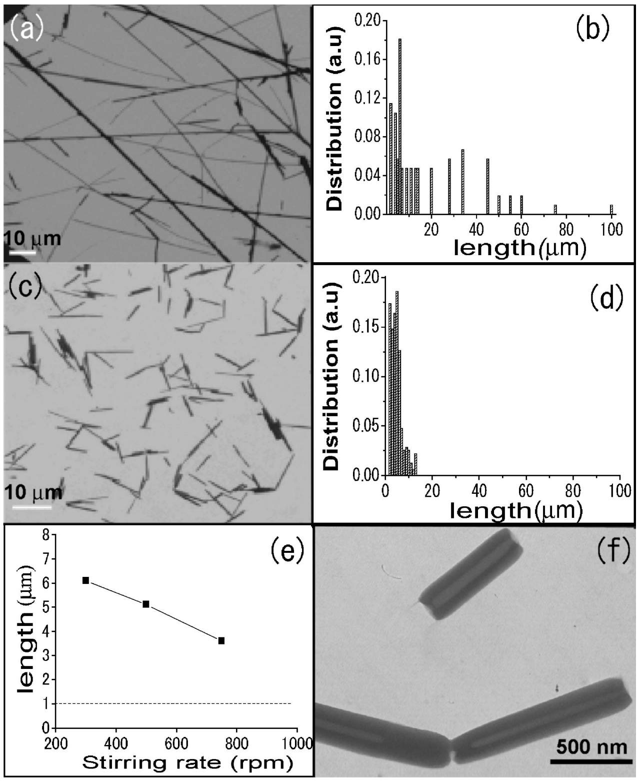
A novel glycolipid, *N*-(11-*cis*-octadecenoyl)-β-D-glucopyranosylamine self-assembles in water to provide monodispersed and well-defined glycolipid nanotubes (LNTs) with a high-axial-ratio structure. Mechanical stirring of the LNT suspension proved to convert the obtained long LNTs into short ones ($<10\,\mu$m) in length, depending on the feature of hydrogen bond network in the LNTs.

Recently, nanostructures formed by self-assembly of organic molecules (e.g. lipid, surfactants, peptide, etc.) are of considerable interest since they have potential applications in materials science and biotechnology.[1–5] The morphology and the size of self-assembled nanostructures are basically correlated with starting molecular structures. Part of the self-assembled nanostructures provides high-axial-ratio nanostructures (HARNs).[6,7] For example, the length of glycolipid nanotubes (LNTs) ranges from several tens µm to several mm. However, the release rate of encapsulated substances depends on the LNT length in controlled release system. Therefore, controlling the LNT lengths should be of great scientific and technological importance. Middle- ($1\,\mu\text{m} < L < 10\,\mu\text{m}$) and short-length LNTs ($<1\,\mu$m) will provide connectors and components for sensor devices, respectively. Several groups have reported how to control the length of HARNs.[8] All these researches have focused on changing the conditions of the self-assembly process such as mixed solvent,[8a] cooling rate,[8b] and addition of metal salt.[8c] To date all approaches have failed in obtaining short-length nanostructures. In this communication, we report a simple approach to obtain the middle and short LNTs using a mechanical stirring system.

In this work, we used a structurally optimized glycolipid, *N*-(11-*cis*-octadecenoyl)-β-D-glucopyranosylamine **1** to form the LNTs since the self-assembly in water can produce mono-dispersed and well-resolved LNTs in approximately 100% yields. The morphologies and the size dimensions of the LNTs were observed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The obtained LNTs possess high-axial-ratios (>1000) and the lengths are in the range of several tens to hundreds µm. The TEM observation clearly indicates the presence of hollow cylinder tubular structures terminated with completely opened two ends. The inner and outer diameter distributions of the LNTs are very narrow, ca. 50 nm for the inside and ca. 200 nm for the outside.

To shorten the length of the long LNTs, we firstly tried the cutting of the LNTs by sonication technique, which has been widely used to shorten carbon nanotubes (CNTs).[9] However, we found this approach unsuitable for the soft LNTs because of the destruction of a lot of LNTs. Thus, we developed a more mild and effective method by means of a mechanical stirring system,[10] and confirmed the shortening effect by TEM observation. When we used water-filled LNTs, we found that the LNTs can be cut to shorten by stirring probably due to their moderate mechanical properties.[11,12]

Figures 1a–1d showed typical TEM images and the distribution diagram for the length of the LNTs after stirring the suspension at 500 rpm for 10 min or 6 h. As shown in these figures, short stirring time (10 min) produced relatively longer LNTs, whereas 6 h stirring gave middle-length LNTs ($1 < L < 10\,\mu$m). The LNTs lengths proved to be controllable by varying two factors: mechanical stirring time and stirring rate. By increasing the stirring time, we obtained middle-length LNTs (Figures 1a–1d). On the other hand, the middle-length LNTs also



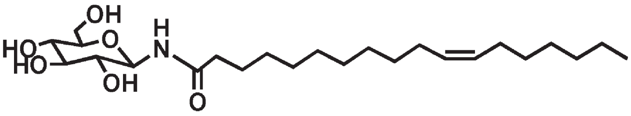


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Figure 1. (a)–(d) TEM images and the length distribution of the shortened LNTs at different stirring time at 500 rpm. (a), (b) after 10 min; (c), (d) after 6 h. (e) plot of average length of shortened LNTs vs. stirring rate. (f) TEM image of lyophilized LNTs after stirring for 10 min at 500 rpm.

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can be obtained by increasing stirring rate. Figure 1e shows the change in average lengths of LNTs after stirring for 6 h at different stirring rate. However, we found that no shorter LNTs (<1 µm) are obtainable by this way even if we take 12 h or at 1000 rpm. Then we tried to apply lyophilized LNT powders for the stirring system and eventually realized remarkable reduction in length of LNTs.[13] Figure 1f displays a high-magnification TEM image of the cut LNTs under the condition of stirring rate (500 rpm) and stirring time (10 min). The LNTs can be cut effectively to shorten by stirring. We, thus, obtained the shortest LNTs (600–800 nm). The shortened lipid nanotubes (LNTs) are stable. We didn't find the increase in length after shortening **1** month in both these case of water-filled and lyophilized LNT.

We found that the lyophilized LNTs can be cut shorter and more quickly than the water-filled LNTs under the same stirring condition (Figures 1a and 1f). This result suggests that the lyophilized LNTs are so labile as to shorten due to the destruction of hydrogen-bond network in the lipid bilayers. Bound water associated with interlayer hydrogen-bond network between lipid bilayers seems to strongly contribute to the stabilization of LNTs.[14] The FTIR experiment supported this suggestion (Figure 2a). The IR spectra of water-filled LNTs showed a sharp peak at 3200 cm^{-1} due to the hydrogen-bonded OH stretching of β-D-glucopyranosylamine molecule with water in LNTs. However, in the IR spectra of the lyophilized LNTs, this peak disappeared. This means that the water is removed through lyophilization. Therefore, the interlayer hydrogen-bond network between lipid bilayers is destroyed to facilitate the further shortening of LNTs (Figure 2b).

In summary, the present mechanical stirring of lyophilized LNTs can provide a very simple and an efficient method to shorten LNTs. This approach can also be applicable to other high-axial-ratio organic nanostructures.

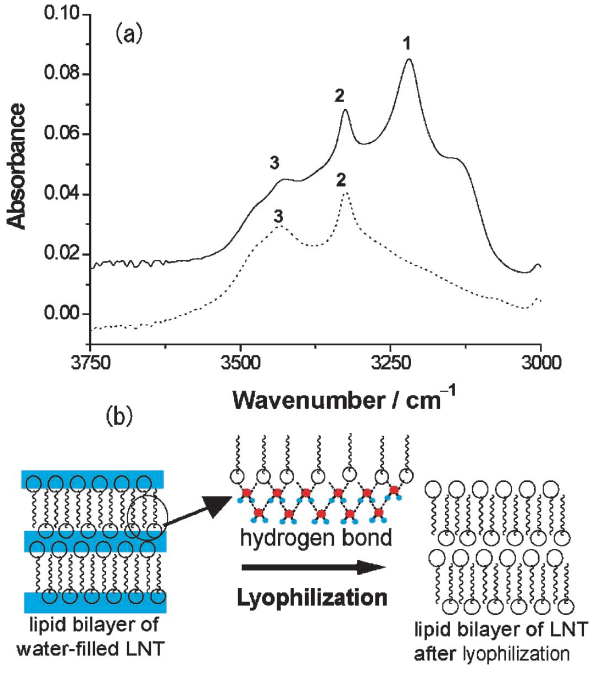

Figure 2. FTIR spectra of water-filled LNTs (solid line) and lyophilized LNTs (dotted line). 1: OH stretching of lipid **1** with bound water; 2: NH stretching of lipid **1**; 3: OH stretching of lipid **1**.

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10 This system consists of a stirred cell with 50 mL volume (MILLIPORE, model 8050) and a hand-held disperser (Model T8, ALDRICH). In a typical procedure, the 20 mL suspension of the LNTs (1 mg) was added in the cell, and the suspension was stirred with the hand-held disperser at 25 °C. The LNTs were found to have a relatively higher gel-to-liquid crystalline phase transition temperature (72 °C), The shortening experiment was, therefore, carried out with keeping the temperature at 25 ± 1 °C using water cooling system.
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